SAURAUIA SPECIES AND THEIR CHROMOSOMES[1]

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Saurauia is a widespread genus of flowering plants, about 250 species of which have been described (Melchior, 1964). The members are represented in both Old and New World tropics and subtropics. The American range extends from Central Mexico in the north to Central Bolivia in the south, through Andean South America. It is not known from the West Indies, and no records indicate its presence in the Guianas and Brazil. According to a recent study by Hunter (1966), 22 species occur in Mexico and Central America, and my present study indicates that 49 species are represented in South America.

The cytology of the genus has never been studied. In his treatment of the Peruvian species of *Saurauia*, Macbride (1955) remarks that ". . . specific characters to this day are not understood and a satisfactory treatment will probably be possible only with cytological as well as morphological data." Hunter (1966), without the benefit of cytological data, was able to show that the species (Mexican and Central American) could be classified comprehensibly on a morphological basis alone. As a non-cytologist, I was stimulated by Macbride's remark, and I set to work to collect cytological "pickles" in the field during the summer of 1963 in the Nariño and Putumayo regions of Colombia. Later, upon examination in the laboratory, the materials which were thought to be in the right developmental stages for the study of meiosis of the pollen mother cells turned out to be very old. Provoked by this failure I collected more cytological materials repeatedly from various parts of South America (Colombia, Ecuador, Peru) during the summers of 1964 and of 1965 and in May and June of 1966 in Colombia. It is from these more adequate materials that the observations here reported were made.

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[1] This paper is a minor part of a thesis ("Studies of South American *Saurauia*") submitted to the Department of Biology of Harvard University in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

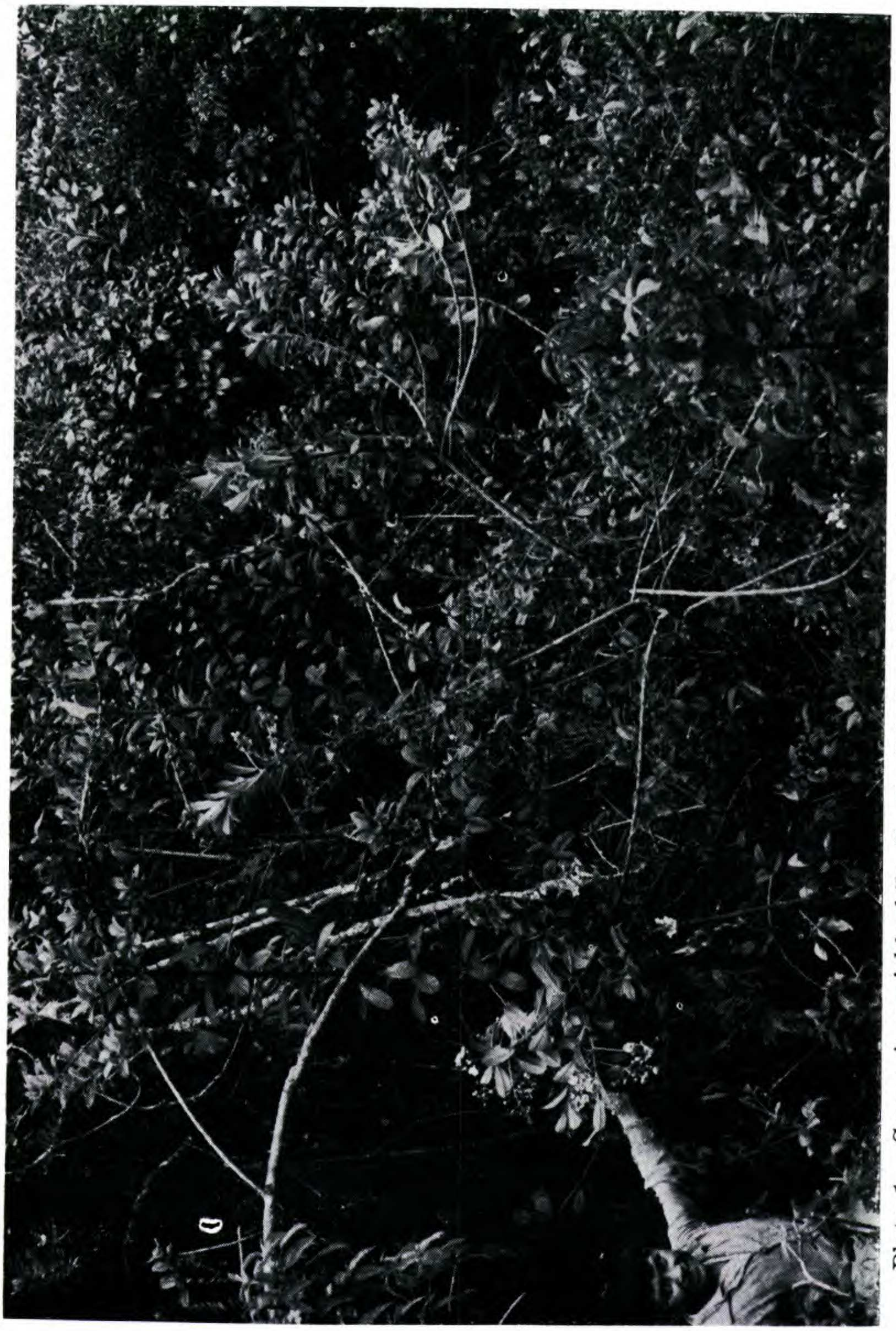

Plate 1. *Saurauia omichlophila* R. E. Schult. (shrub with white flowers, which the boy is touching). Nariño, above Pasto, Bosque Botana, humid mountain forest, alt. ca. 3000 m.

Plate 2. *Saurauia bullosa* Wawra. Nariño, above Pasto, Bosque Botana, second growth vegetation, alt. ca. 2800 m. Note the wheel-like pattern of insertion of the primary branches.

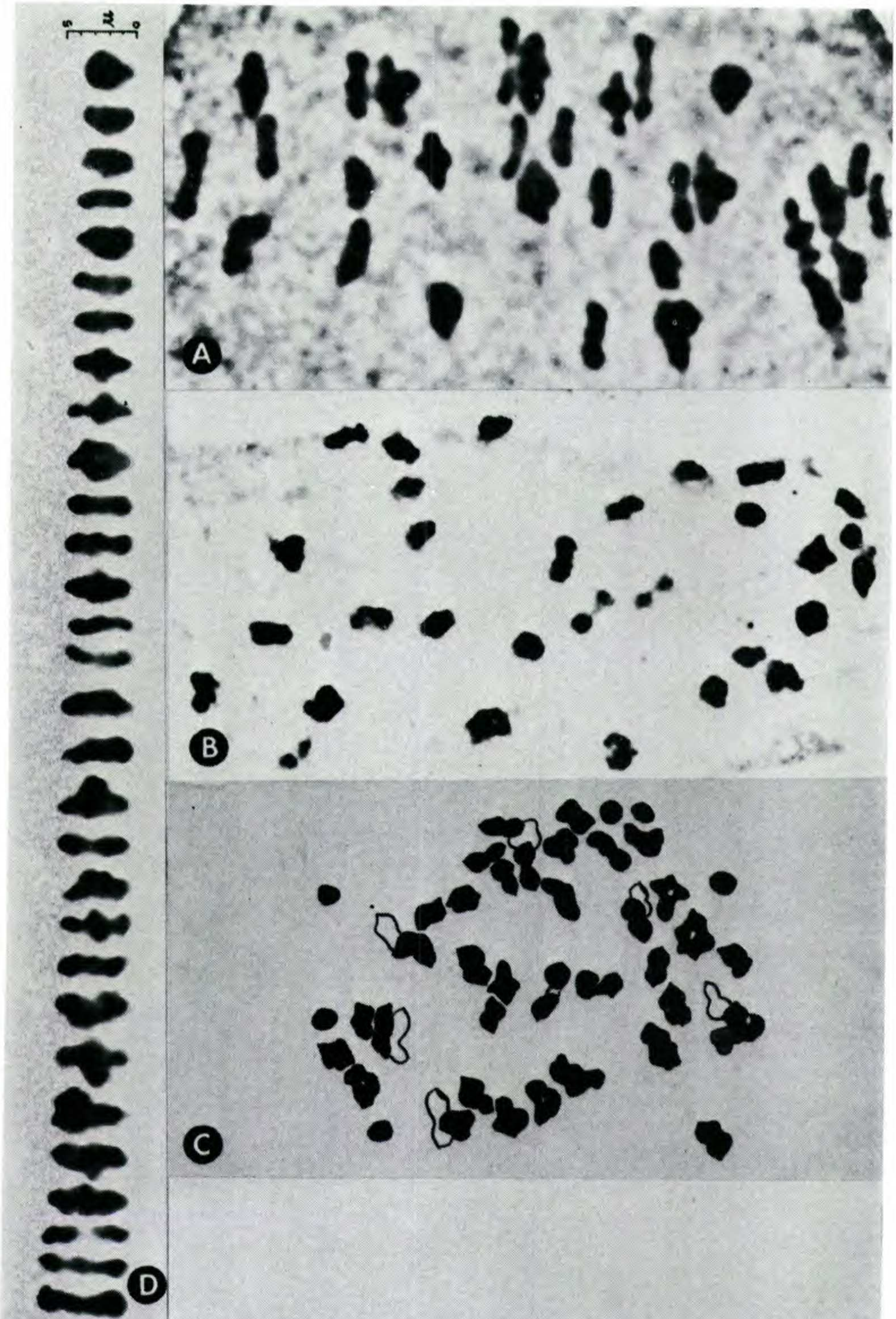

Plate 3. Meiotic configurations in *Saurauia*. A, *S. bullosa*, early anaphase I, equatorial view, $n=30$. B, *S. omichlophila* (voucher: *Soejarto 914*), diakinesis, $n=30$. C, *S. isoxanthotricha* (voucher: *Soe-*

MATERIALS AND METHODS

Flower buds at different stages of development were collected in the field at various times of the day and were killed and fixed immediately in Carnoy's solution (3 parts absolute alcohol, 1 part glacial acetic acid by volume) for from 24 to 72 hours. Since the sepals in most species form a thick cover over the bud, these were first removed or, when the buds are small, were cracked in several places (without removing the sepals), before dipping them into the fixative so that the liquid could penetrate the tissue rapidly. Small vials were used as containers. At the end of the period indicated above, the fixative was substituted by 70% ethyl alcohol. Due to the nature of travel, no serious attempt was made to keep these vials in refrigeration. Only after arrival in the United States, were these vials containing the fixed materials kept in the refrigerator until ready for examination.

For each cytological specimen, a voucher was collected. A complete set of the vouchers is deposited at the Gray Herbarium of Harvard University. Permanent microscope slides are deposited in the Harvard Botanical Museum.

RESULTS

Nineteen individuals representing fifteen species have been observed and the chromosomes counted. The chromosome behavior at meiosis in all but one of the individuals studied appears normal, and the chromosome morphology and number are remarkably stable. Representative meiotic configurations are shown on Plate 3 and Plate 4. The gametic chromosome number of the fifteen species observed is $n = 30$, and the chromosome size during the first metaphase (meta-anaphase) varies but little, as shown by the cut-ups on Plate 3. The chromosome length at this stage ranges from 3.5-6.5μ, the mean length being 5μ.

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jarto 1563), diakinesis, n=45 (42*II* + 6*I*). D, cut-ups of A arranged linearly to show size range and variation of the early anaphase (meta-anaphase) chromosomes. All the same scale, approx. 1600×. A and B photomicrographs, C camera lucida drawing.

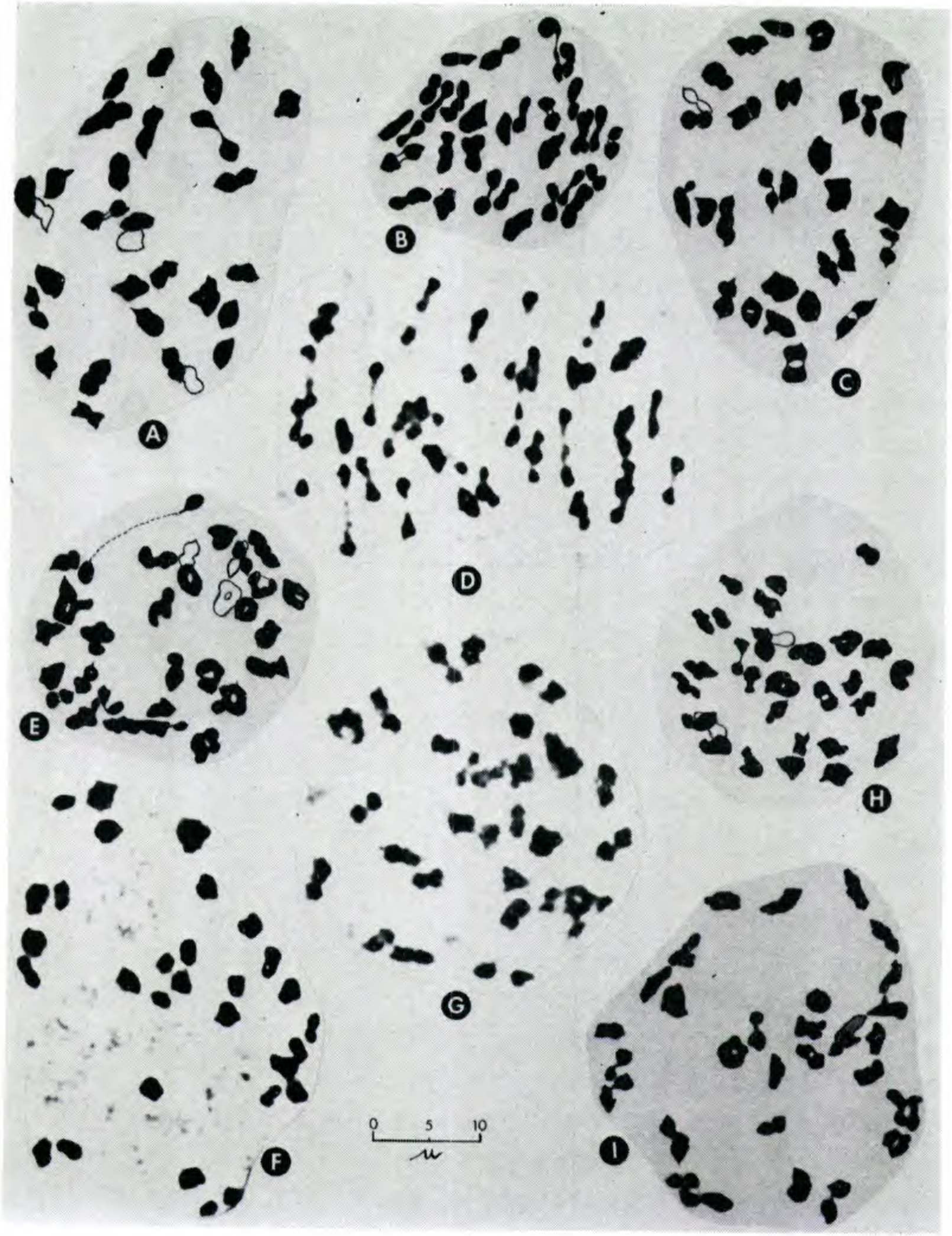

Plate 4. Meiotic configurations in *Saurauia*. A, *S. biserrata* (*Soejarto 1426*). B, *S. Stapfiana* (*Soejarto 2046*). C, *S. pastasana* (*Soejarto 1344*). D, *S. tomentosa* (*Soejarto 1433*). E, *S. brachybotrys* (*Soejarto 2052*). F, *S. Humboldtiana* (*Soejarto 913*). G, *S. aequatoriensis* (*Soejarto 1342*). H, *S. isoxanthotricha* (*Soejarto 1531*). I, *S. portachuelensis* (*Soejarto 1158*). All about 1600×. A, B, C, E, H camera lucida drawings; D, F, G photomicrographs; I a tracing of a photomicrograph.

In the course of the study, it was noted that the size of the pollen mother cells is relatively stable in all the nineteen individuals the diameter being 35-50μ. A slight increase in diameter is observed in *Soejarto 1563*, the diameter being 45-60μ. For technical reasons, no detailed study of the meiotic process was attempted, yet some remarks regarding the successive stages observed during the chromosome counts are given below.

The size of the flower buds in which meiosis was observed is not constant in all the species studied; this is due, primarily, to the different sepal thickness and the stamen number in the various species. To cite examples: the flower buds in which meiosis was observed for *S. bullosa* and *S. tomentosa* (with 100-200 stamens and relatively thick sepals) have a diameter of about 10 mm, whereas for *S. chiliantha* and *S. portachuelensis* (with 15-30 stamens and relatively thin sepals) the diameter is about 3 mm. However, anther size, in which meiosis was observed, is essentially the same, being 0.5-1.0 mm in length and about 0.25-0.5 mm in width. Examination of various anthers from the same flower bud from which meiosis was observed shows that the developmental stages within one bud are not uniform. The outer whorls of the anthers (especially those species with high stamen number) were observed to show earlier stages of meiosis than those of the inner whorls. Although I made no quantitative study, I believe that this phenomenon agrees with Brown's study (1935) of the centrifugal maturation of the stamens in *Saurauia subspinosa*.

The pollen mother cells are globose to subglobose and at the resting stage each possesses a rather large nucleus, containing one or two nucleoli (when only one, it is either centric or excentric). During the early phases of meiotic prophase, there is an increase in cell size (from about 30μ to 40-50μ), and the chromosomes appear as densely intertwined threads which are very unsuitable for analysis. Chiasma formation begins to be seen in diplotene rather clearly, but the materials are not favorable for the exact determination of its frequency, both in this stage as well as in later stages; furthermore, the chromosomes in this stage appear slightly too diffuse for an exact delimitation of the chiasmata. At diakinesis, the chromosomes attain the greatest contraction, and staining reaction with acetocarmine is good. They appear quite dark and are distributed rather evenly in the cell, which facilitates counting. Both ring and rod bivalents were observed, but they tend to appear as dark blobs, making exact determination difficult. The cytoplasm in most species examined also takes stain, though lightly, which occasionally renders correct counting unfavorable. In some species, such as *S. peduncularis*, the nucleolus is quite late in disappearing, but in the majority, it becomes invisible during diakinesis. At the first metaphase, the chromosomes become arranged on the equatorial plane which is usually impossible to analyze from a lateral view, and the spindle fibers are only barely discernible. As a result of squashing, the chromosomes in the early first anaphase (meta-anaphase) frequently become well separated (especially in *S. tomentosa* and *S. bullosa*) and good for counting, above all when the pollen mother cells are well separated as a result of thorough maceration. Countings reported here were made either from diakinesis and/or early first anaphase configurations, at an average of 15 cells per preparation. During the early first anaphase, the bivalents are held together by one or two terminal chiasmata or one interstitial chiasma. In this stage, the position of the centromeres could also be observed, either median to submedian or subterminal, the majority being subterminal. No lagging chromosomes were observed during the first anaphase in the individuals with $n = 30$, though, very rarely, bridge formations were noted. In *Soejarto 1563*, however, the univalents usually move to the poles earlier than the bivalents, though occasionally they lag behind; bridge formation was also observed. It should be noted that at the metaphase plate, in the case of *Soejarto 1563*, the univalents are distributed randomly. At the dyad stage, presumably at the beginning of homotypic division, the chromosomes are sometimes distributed rather evenly to facilitate counting.

At the second metaphase and anaphase, the two sets of the spindle fibers lie either in the same plane or at right angles to each other. Finally, cytokinesis is of simultaneous type and the tetrad arrangement of the pollen grains is tetrahedral.

The developmental stage within a single anther is not uniform (synchronous) in all the species examined; although usually one or two meiotic stages are represented, very frequently more were observed, e.g., diakinesis is observed among the numerous pollen mother cells, either in the first metaphase and/or anaphase, even in telophase to tetrad stage. The only explanation which I can offer is that this phenomenon may have been the result of the different collecting time of the specimens. In all the individuals studied, I always observed pieces or complete packets of rhaphides among the pollen mother cells. Presumably, these are present within the anther tissue and became broken and separated during maceration and squashing.

The results of chromosome counts are summarized in the following Table, where the order of the species is listed geographically, in a north-to-south and west-to-east order, corresponding alphabetically to the order of the species in the accompanying map.

DISCUSSIONS AND CONCLUSIONS

This study has made clear that chromosome number and morphology in *Saurauia* appear to be of little use in specific classification and grouping. At least 14 species have highly similar chromosome morphology, with gametic chromosome complement of $n = 30$. However, any statement at the present stage of our knowledge should be considered tentative, and a final conclusion must await further study, preferably including comparative data of representatives from Central America, Mexico, and Asia.

The existence of an individual with a gametic chromosome number of $n = 45$ (39-42 bivalents plus 12-16 univalents) can be explained as a result of interspecific hybridization. Mitotic figures obtained from root tip smear

SPECIES	VOUCHER SPECIMEN	ORIGIN	ALTITUDE (m.)	MEIOTIC PAIRING
A. *S. brachybotrys* Turcz.	Soejarto 2052	Colombia, Dpto. Antioquia, Medellin, Sta. Helena	2000	30*II*
B. *S. ursina* Tr. & Pl.	Soejarto 2040	Colombia, Dpto. Antioquia, Las Palmas	2000	30*II*
C. *S. Stapfiana* Busc.	Soejarto 2045 2046	Colombia, Dpto. Antioquia, Sonsón	2800	30*II*
D. *S. chiliantha* R.E. Schult.	Soejarto 2047	Colombia, Dpto. Antioquia, Sonsón	2800	30*II*
E. *S. Humboldtiana* Busc.	Soejarto 913	Colombia, Dpto. Cundinamarca Salto de Tequendama	2700	30*II*
F. *S. peduncularis* Tr. & Pl.	Soejarto 938, 1456	Colombia, Dpto. Nariño, Río Guasa	1500	30*II*
G. *S. omichlophila* R.E. Schult.	Soejarto 914, 1493	Colombia, Dpto. Nariño, Bosque Botana	3000	30*II*
H. *S. isoxanthotricha* Busc.	Soejarto 1531	Colombia, Com. Putumayo, Cro. Portachuelo	2300	30*II*
S. isoxanthotricha Busc.	Soejarto 1563	Colombia, Com. Putumayo, Cro. Portachuelo	2300	(39*II*-42*II*) + (12*I* -6*I*)
I. *S. bullosa* Wawra	Soejarto 1473	Colombia, Dpto. Nariño, Bosque Botana	2900	30*II*
J. *S. tomentosa* (HBK.) Spreng.	Soejarto 1433	Colombia, Dpto. Nariño, El Encano	2800	30*II*
K. *S. portachuelensis* R.E. Schult.	Soejarto 1158	Colombia, Com. Putumayo, Cro. Portachuelo	2700	30*II*
L. *S. putumayonis* R.E. Schult.	Soejarto 1573	Colombia, Com. Putumayo, Cro. Portachuelo	2300	30*II*
M. *S. aequatoriensis* Sprague	Soejarto 1342	Ecuador, Prov. Tungurahua, Baños	1800	30*II*
N. *S. Prainiana* Busc.	Soejarto 1344	Ecuador, Prov. Napo-Pastaza, Shell Mira	1300	30*II*
O. *S. biserrata* (R. & P.) Spreng.	Soejarto 1426	Perú, Dpto. Huanuco, above Chinchao	2500	30*II*

TABLE I. Results of chromosome counts.

Agricola of Universidad de Nariño, and Dr. Alvaro Fernández P., director of the Botany Department, Instituto Ciencias Naturales, Universidad Nacional, Bogotá.

Finally, I wish to thank my wife, Mariela, for her constant encouragement, and for typing the manuscript. Any errors and/or misinterpretations found in this paper, however, are my sole responsibility.

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ADDITIONAL CHROMOSOME NUMBERS IN BRAZILIAN COMPOSITAE

JAMES R. COLEMAN

While serving as a research taxonomist at the Instituto de Botânica of São Paulo, Brazil, I had the opportunity to collect material of Brazilian Compositae for cytological study. A previous report on this study has been published (Coleman 1968).

In the present paper 40 counts are reported for 35 species of 18 genera (Table 1). Initial reports are given for 29 species and 2 genera. The initial generic reports are for *Elvira*, $n = 12$, and *Ichthyothere*, $n =$ ca 33.

The methods used are identical to those described by Coleman (1968). The material of *Eupatorium ballotaefolium*, *E. organense* and *E. tremulum* was determined by Dr. Lyman B. Smith and *Simsia dombeyana* by Dr. J. Cuatrecasas. My appreciation is sincerely expressed. All other identifications are my own. A complete set of voucher specimens is deposited in the University of Georgia Herbarium and a nearly complete set in the Herbarium of the Instituto de Botânica of São Paulo, Brazil.

VERNONIEAE

The report of $n = 16$ for *Centratherum punctatum* agrees with an earlier report for that species based on Panamanian material (Turner and King 1964). No other counts have been reported for the genus.

EUPATORIEAE

The count of $n = 10$ for *Ageratum campuloclinoides* is the initial report for the species. Robinson (1913) considered *A. campuloclinoides* a doubtful *Ageratum* and suggested its affinities to be with *Trichogonia*. Since the counts reported to date for *Trichogonia* agree with those of *Ageratum* in being based on $x = 10$, chromosome number is no aid in better defining the relationship of *Ageratum campuloclinoides*.

The report of $n = 10$ for *Alomia fastigiata* agrees with a previous report for this species (Coleman 1968). The Central American *Alomia microcarpa* (Benth.) Rob. has also been reported as $n = 10$ (Turner and King 1964). Counts of $n = 9$ are reported for two populations of *Alomia longifolia* (Gardn.) Rob. This species was originally described in the genus *Ageratum*, but was subsequently transferred to *Carelia* and later *Alomia*. No reports are available for *Carelia*, but previous reports for both *Ageratum* and *Alomia* are based on $x = 10$.

North American species of *Stevia* have been reported as $n = 11$, 12 and 17 (Powell and Turner 1963). With the report of $n = 12$ for *Stevia organensis*, counts of $n = 11$ and 12 have now been reported for Brazilian species.

The count of $n = 10$ for *Eupatorium laetevirens* agrees with an earlier report for the species. The report of $n = 10$ for *E. ballotaefolium* concurs with a count presented for Colombian material of that species (Powell and King, 1969). I have previously reported $n = 30$ for *E. ballotaefolium* from southern Brazil (Coleman, 1968).

ASTEREAE

Counts of $n = 9$ for species of *Baccharidastrum* and *Baccharis* are in agreement with previous reports for these genera.

HELIANTHEAE

Blainvillea rhomboidea, $n = 17$, is the second species of the genus to be reported. *Blainvillea latifolia* DC. has been reported as $n = 39$ (Mehra et al 1965). An approximate count of $n = 24 \pm 2$ is reported for *Spilanthes acmella*. Previous counts of $n = 7$ (Malik and Ahmad in Cave 1964) and $n = 12$ (Mehra et al 1965) have been reported. Turner et al (1962) have shown a diploid, tetraploid and hexaploid series to exist in *S. americana* Hieron. A similar situation evidently occurs in *S. acmella*.

The counts of $n = 12$ for *Elvira biflora* and $n =$ ca 33 for *Ichthyothere rufa* are the initial reports for these genera.